PATENT
Attorney Docket No. 3495.0111-12

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	
	)	
Bernard DUJON et al.	)	**Prior application: 09/196,131**
	)	
Serial No.: To be assigned	)	Group Art Unit: Unknown
	)	
Filed: April 18, 2001	)	Examiner: Unknown

For: NUCLEOTIDE SEQUENCE ENCODING
THE ENZYME I-SCEI AND THE USES THEREOF

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

PRELIMINARY AMENDMENT

Prior to the examination of the above application, please amend this application as follows:

IN THE SPECIFICATION:

On page 5, replace the paragraph beginning on line 4 with the following new paragraph:

-- Accordingly, this invention aids in fulfilling these needs in the art. Specifically, this invention relates to an isolated DNA encoding the enzyme I-*Sce*I. The DNA has the following nucleotide sequence:

```
                                        ATG CAT ATG AAA AAC ATC AAA AAA AAC CAG GTA ATG 2670
                                        M   H   M   K   N   I   K   K   N   Q   V   M   12

2671 AAC CTC GGT CCG AAC TCT AAA CTG CTG AAA GAA TAC AAA TCC CAG CTG ATC GAA CTG AAC 2730
  13 N   L   G   P   N   S   K   L   L   K   E   Y   K   S   Q   L   I   E   L   N   32

2731 ATC GAA CAG TTC GAA GCA GGT ATC GGT CTG ATC CTG GGT GAT GCT TAC ATC CGT TCT CGT 2790
  33 I   E   Q   F   E   A   G   I   G   L   I   L   G   D   A   Y   I   R   S   R   52

2791 GAT GAA GGT AAA ACC TAC TGT ATG CAG TTC GAG TGG AAA AAC AAA GCA TAC ATG GAC CAC 2850
  53 D   E   G   K   T   Y   C   M   Q   F   E   W   K   N   K   A   Y   M   D   H   72
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2851 GTA TGT CTG CTG TAC GAT CAG TGG GTA CTG TCC CCG CCG CAC AAA AAA GAA CGT GTT AAC 2910
  73 V   C   L   L   Y   D   Q   W   V   L   S   P   P   H   K   K   E   R   V   N   92

2911 CAC CTG GGT AAC CTG GTA ATC ACC TGG GGC GCC CAG ACT TTC AAA CAC CAA GCT TTC AAC 2770
  93 H   L   G   N   L   V   I   T   W   G   A   Q   T   F   K   H   Q   A   F   N   112

2971 AAA CTG GCT AAC CTG TTC ATC GTT AAC AAC AAA AAA ACC ATC CCG AAC AAC CTG GTT GAA 3030
 113 K   L   A   N   L   F   I   V   N   N   K   K   T   I   P   N   N   L   V   E   132

3031 AAC TAC CTG ACC CCG ATG TCT CTG GCA TAC TGG TTC ATG GAT GAT GGT GGT AAA TGG GAT 3090
 133 N   Y   L   T   P   M   S   L   A   Y   W   F   M   D   D   G   G   K   W   D   152

3091 TAC AAC AAA AAC TCT ACC AAC AAA TCG ATC GTA CTG AAC ACC CAG TCT TTC ACT TTC GAA 3150
 153 Y   N   K   N   S   T   N   K   S   I   V   L   N   T   Q   S   F   T   F   E   172

3151 GAA GTA GAA TAC CTG GTT AAG GGT CTG CGT AAC AAA TTC CAA CTG AAC TGT TAC GTA AAA 3210
 173 E   V   E   Y   L   V   K   G   L   R   N   K   F   Q   L   N   C   Y   V   K   192

3211 ATC AAC AAA AAC AAA CCG ATC ATC TAC ATC GAT TCT ATG TCT TAC CTG ATC TTC TAC AAC 3270
 193 I   N   K   N   K   P   I   I   Y   I   D   S   M   S   Y   L   I   F   Y   N   212

3271 CTG ATC AAA CCG TAC CTG ATC CCG CAG ATG ATG TAC AAA CTG CCG AAC ACT ATC TCC TCC 3330
 213 L   I   K   P   Y   L   I   P   Q   M   M   Y   K   L   P   N   T   I   S   S   232

3331 GAA ACT TTC CTG AAA TAA  (SEQ ID NO:1)
 233 E   T   F   L   K   *    (SEQ ID NO:2). --
```

On page 7, beginning on line 2 and ending at the bottom of the page, replace paragraphs 1-11 with the following new paragraphs:

-- This invention will be more fully described with reference to the drawings in which:

Fig. 1 depicts the universal code equivalent of the mitochondrial I-*Sce*I gene (SEQ ID NO:1).

Fig. 2 depicts the nucleotide sequence of the invention encoding the enzyme I-*Sce*I and the amino acid sequence of the natural I-*Sce*I enzyme (SEQ ID NOS: 5 and 2).

Fig. 3 depicts the I-*Sce*I recognition sequence and indicates the possible base mutations in the recognition site and the effect of such mutations on stringency of recognition (SEQ ID NOS: 6, 7, and 8).

Fig. 4 is the nucleotide sequence and deduced amino acid sequence of a region of plasmid pSCM525. The nucleotide sequence of the invention encoding the enzyme I-*Sce*I is enclosed in the box (SEQ ID NOS: 9 through 16).

Fig. 5 depicts variations around the amino acid sequence of the enzyme I-*Sce*I (SEQ ID NO: 2).

Fig. 6 shows Group I intron encoding endonucleases and related endonucleases (SEQ ID NOS: 17-44).

Fig. 7 depicts yeast expression vectors containing the synthetic gene for I-SceI.

Fig. 8 depicts the mammalian expression vector PRSV I-*Sce*I.

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Fig. 9 is a restriction map of the plasmid pAF100. (See also YEAST, 6:521-534, 1990, which is relied upon and incorporated by reference herein).

Figs. 10A and 10B show the nucleotide sequence and restriction sites of regions of the plasmid pAF100 (SEQ ID NOS: 45-50). --

On page 12, replace the last paragraph with the following new paragraph:

-- The enzyme I-*Sce*I has a known recognition site. (ref. 14.) The recognition site of I-*Sce*I is a non-symmetrical sequence that extends over 18 bp as determined by systematic mutational analysis. The sequence reads: (arrows indicate cuts)

```
                ↓
5'   TAGGGATAACAGGGTAAT   3' (SEQ ID NO:51)
3'   ATCCCTATTGTCCCATTA   5' (SEQ ID NO:52). --
                ↑
```

On pages 41 to 42, replace the bridging paragraph with the following:

-- -e- The supernatant of this clone was used to infect other mouse cells (1009) by spreading 10^5 virus particles on 10^5 cells in DMEM medium with 10% fetal calf serum and 5 mg/ml of "polybrene" (hexadimethrine bromide). Medium was replaced 6 hours after infection by the same fresh medium. --

After page 52, and before page 53, please insert the attached pages titled "SEQUENCE LISTING".

IN THE CLAIMS

Please cancel claims 1-26.

Please add the following new claims:

--27. A method for *in vivo* site directed genetic recombination in an organism comprising:

(a) providing a transgenic cell having at least one HO endonuclease or Group I intron encoded endonuclease recognition site inserted at a unique location in a chromosome;

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(b) providing an expression vector that expresses said endonuclease in said transgenic cell;

(c) providing a plasmid comprising a gene of interest and a DNA sequence homologous to the sequence of the chromosomal DNA, allowing homologous recombination;

(d) transfecting said transgenic cell with said plasmid of step (c);

(e) expressing said endonuclease from said expression vector in said cell; and

(f) cleaving said endonuclease recognition site with said endonuclease, whereby said cleavage promotes the insertion of said gene of interest into said chromosome of said organism at a specific site by homologous recombination.

28. The method of claim 27, wherein said endonuclease recognition site has been introduced into said cells by homologous recombination.

29. The method of claim 27, wherein said endonuclease recognition site has been introduced into said cells by retroviral insertion.

30. The method of claim 27, wherein said organism is yeast.

31. The method of claim 27, wherein said organism is bacteria.

32. The method of claim 27, wherein said organism is a mammal.

33. The method of claim 27, wherein said endonuclease site is a Group I intron encoded endonuclease site.

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34. The method of claim 33, wherein said endonuclease recognition site is selected from the group consisting of Class I I-endonuclease sites, Class II I-endonuclease sites, Class III I-endonuclease sites, Class IV I-endonuclease sites, and Class V I-endonuclease sites.

35. The method of claim 34, wherein said endonuclease recognition site is a Class I I-endonuclease site.

36. The method of claim 35, wherein said endonuclease recognition site is selected from the group consisting of I-*Sce*I, I-*Sce*IV, I-*Csm*I, and I-*Pan*I sites.

37. The method of claim 36, wherein said endonuclease recognition site is an I-*Sce*I site. --

REMARKS

Entry and consideration of this amendment is respectfully requested.

Claims 1-26 have been canceled. New claims 27-37 find support throughout the specification, for example on pages 20-21 and Fig.6. Accordingly, no new matter is entered by amendment.

Applicants submit herewith a Sequence Listing and have amended the specification to conform with the requirements of 37 C.F.R. §§ 1.821-1.825.

Applicants request the use of the computer-readable form of the Sequence Listing in U.S. Application Serial No. 08/417,226, filed April 5, 1995, now U.S. Patent No. 5,962,327, issued October 5, 1999.

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I hereby state that the contents of the paper copy of the Sequence Listing in this application and the computer-readable form of the Sequence Listing in U.S. Application Serial No. 08/417,226, filed April 5, 1995, submitted in accordance with 37 C.F.R. § 1.821(c) and (e), respectively, are the same.

Attached hereto is a marked-up version of the changes made to the specification by the current amendment. The attached page is captioned "**Version with Markings to Show Changes Made**."

Please grant any extensions of time required to enter this response and charge any additional required fees to our deposit account 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER, L.L.P.

Dated: April 18, 2001

By: _____

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Version with Markings to Show Changes Made

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  13 N   L   G   P   N   S   K   L   L   K   E   Y   K   S   Q   L   I   E   L   N   32

2731 ATC GAA CAG TTC GAA GCA GGT ATC GGT CTG ATC CTG GGT GAT GCT TAC ATC CGT TCT CGT 2790
  33 I   E   Q   F   E   A   G   I   G   L   I   L   G   D   A   Y   I   R   S   R   52

2791 GAT GAA GGT AAA ACC TAC TGT ATG CAG TTC GAG TGG AAA AAC AAA GCA TAC ATG GAC CAC 2850
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  73 V   C   L   L   Y   D   Q   W   V   L   S   P   P   H   K   K   E   R   V   N   92

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3331 GAA ACT TTC CTG AAA TAA  (SEQ ID NO:1)
 233 E   T   F   L   K   *    (SEQ ID NO:2).
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-e- The supernatant of this clone was used to infect other mouse cells (1009) by spreading 10^5 virus particles on 10^5 cells in DMEM medium with 10% fetal calf serum and 5 mg/ml of "[polybrain] polybrene (hexadimethrine bromide)". Medium was replaced 6 hours after infection by the same fresh medium.

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